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Research report

Atropine-induced, state-dependent learning for spatial information, but not for visual cues

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Abstract

This study investigates state-dependent learning employing atropine. The reaction of rats to (1) the presentation of novel stimuli, (2) habituation to intermittent presentations of the same stimulus at the same local, (3) spatial change at the site of stimulus presentation, and (4) a visual stimulus change, was investigated in the straight alleyway test, controlling for the possible development of behavioral and/or pharmacological tolerance. Our findings reveal that rats habituated to stimulus presentation at a specific location, when under an atropine effect, do react to stimulus presentation at another location, or to a different stimulus, when under an atropine effect, indicating that this drug does not interfere with the acquisition of spatial or visual information. Differently, however, rats habituated to stimulus presentation at a specific location in the absence of an atropine effect are unable to react to spatial change when under the atropine effect, but do react to a visual stimulus change. This suggests that atropine interferes either with the retrieval of previously acquired spatial information or with the comparison of previously acquired spatial information with current information, but does not interfere with visual recognition. These findings reveal that atropine interferes with the use of spatial information acquired in the absence of a drug effect.

Keywords: Memory; Hippocampus; Atropine sulfate; Muscarinic cholinergic antagonist; Drug-state dependent; State-dependent learning; Spatial discrimination; Exploratory behavior

1. Introduction

The cholinergic system plays an important role in learning and memory processes. Drugs that impair the function of the central cholinergic system usually impair memory [3,5,6,13,22,23,24,26,27]; conversely, drugs that potentiate central cholinergic function may, under certain circumstances, enhance memory [7,14]. The participation of central cholinergic systems in modulating cognitive functions has received experimental attention in studies on humans [3], monkeys [24] and rats [6,7,12,13,16,26,27]. Most address the enhancing properties of the cholinergic system on spatial memory acquisition and/or retention. A possible cholinergic effect on the retrieval of spatial *versus* non-spatial stored memories, using tasks that impose similar behavioral demands for spatial and non-spatial information, and controlling for pharmacological and behavioral tolerance, has not been considered. In the present study, a state-dependent-learning protocol (see [20], and below) was employed to examine the possible effect of cholinergic blockade on the retrieval of spatial and non-spatial stored memories, using atropine.

State-dependent learning refers to the retrieval of information acquired in the same sensory context and physiological state as that present during encoding ([1,9,20,21,25]). Such learning is commonly characterized in pharmacological studies employing a 2×2 experimental design in which groups of animals are first trained under either a drug (D) or no-drug (N) effect, and then tested for recall under either the same drug (D) or no-drug (N) effect, according to the pairings N-N, N-D, D-N and D-D. Interpretation of the main drug effects depends on the behavioral outcomes (for details, see [20]). For example, poor performance during testing by groups exposed to a drug state change (N-D and D-N) accompanied by normal performance in groups not exposed to a drug state change (N-N and D-D)

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reveals state dependency. Differently, while poor performance during testing by animals receiving a drug before the training session (D-N and D-D) suggests the occurrence of acquisition disruption, poor performance during testing under the drug effect (N-D and D-D) suggests that the drug interferes with the retrieval of previously learned material. In addition, poor testing performance in the N-D condition associated with normal performance in the D-D and D-N conditions may suggest (1) that the drug interferes with the retrieval of information stored in the absence of the drug, and that retrieval of information stored under the drug effect is possible when acquisition occurs under the drug effect, (2) the occurrence of behavioral tolerance, *i.e.*, temporary performance impairments that occur when the drug is first administered, allowing the animal to learn how to deal with the debilitating effect of the drug, or (3) the occurrence of pharmacological tolerance, *i.e.*, after the initial drug application the animals develop drug tolerance that minimizes the effect of a subsequent dose. In the present study, the drug and no-drug administration schedule was planned to control for these possibilities (see below).

The medial septum is known to project cholinergic fibers to the hippocampus; such projection seems to be critically involved in the septo-hippocampal processing of spatial information [2,10,11,18,19,28]. Congruently, the administration of muscarinic cholinergic blockers induces consistent impairments of performance in spatial tasks; to illustrate, the use of atropine, a muscarinic cholinergic antagonist, impairs the performance of rats in the radial arm maze [23], the tree-table maze [5], the traditional water maze [22], and the water T-maze [14]. These findings raise intriguing questions. For instance, are these marked atropine effects specifically related to the use of hippocampus-dependent spatial tasks? Would such atropine effects occur if non-spatial tasks were used? Does atropine interfere with the acquisition or retrieval of spatial information?

O'Keefe and Nadel [18] proposed that the hippocampus provides a cognitive map of the environment. They distinguished alternative strategies used by animals to navigate through the environment, and suggested that more than one strategy may be used simultaneously to solve spatial tasks. According to these authors, while place (or locale) strategies involve cognitive mapping, guidance (or taxon) strategies depend on a particular prominent object or stimulus that indicates the goal location; egocentric orientation strategies are based on the rotation of the body axis relative to other axes. O'Keefe and Nadel [18] postulated that such strategies would be served by different neural systems; the hippocampus would be necessary for place learning.

According to this view, hippocampal damage should disrupt place discrimination while sparing discriminations that do not require the place dimension. Xavier et al. [30] evaluated this hypothesis by testing rats with dorsal hippocampectomy in a behavioral task that enabled assessment of their ability to deal with either spatial or non-spatial information, but whose response requirements are the same for both types of information. The animals were trained to run a shuttle-alleyway for food up to an assymptotic level of performance. Subsequently, several testing sessions were run to evaluate (1) exploratory behavior directed to the place at which novel, distracting visual stimuli (black cards on the walls) were presented in the alleyway, (2) the reduction in exploratory activity (habituation) to the intermittent presentation of the same stimulus at the same location, (3) reaction to presentation of the same stimulus (to which the rats had become habituated) at a novel location in the alleyway, and (4) reaction to the presentation of a different stimulus (black and white checkered cards on the walls, instead of black cards) at the location where the stimulus had been presented previously (and to which the rats had become habituated). The findings were straight-forward; like the controls, hippocampectomized rats did explore the black cards, did habituate to intermittent presentation of this stimulus at the same location, and did react to its substitution by the black and white checkered cards, indicating that damage to the hippocampus does not disrupt ability to explore novelty, to habituate to repetitive presentations of the same stimulus, or to compare the representation of a previously presented stimulus, stored in memory, with a current novel stimulus. In contrast, and differing from their controls, rats with damage to the hippocampus do not react to the location change for stimulus presentation, suggesting that the ability to compare a previous location of stimulus presentation, which for control rats was stored in memory, with a current one, was disrupted in hippocampectomized rats.

Xavier et al. [29] emphasized some of the advantages of the straight alleyway task in investigating cognitive functions. The fact that the behavioral response is unrelated to the reinforcement but, rather, competes with it renders this response a good index of exploratory activity. In addition, the exact same response is measured for the different cognitive functions under evaluation; thus, impairments following only one specific manipulation cannot be ascribed to the behavioral output. Finally, as stated by Xavier et al. [29], "both extra- and intra-maze cues can be manipulated either alone or in association, allowing tests of rats' capacity (1) to code external events, store this information in the form of a representation, and pay particular attention to places that are changing; and (2) to detect and react to spatial and directional-contextual changes independently of their own direction of locomotion in the maze." (p. 172).

The purpose of the present experiments was to associate the protocol for state-dependent learning using atropine with the testing of rats in the straight alleyway task to evaluate whether cholinergic blockade interferes with either the acquisition or retrieval of information, and whether similar effects occur when spatial and non-spatial information are processed. A 2×2, state-dependent, learning experimental design was used, including the pairings N-N, N-D, D-N and D-D, both to detect a change in the location of stimulus presentation and to detect a change in the visual pattern of the stimulus. These detections respectively require comparison between a previous location of stimulus presentation with a current one, and comparison between a previously presented stimulus and a current one. The experimental design controlled for the possible occurrence of behavioral and/or pharmacological tolerance.

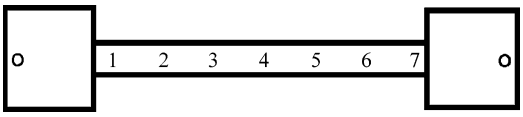

Fig. 1. A top view of the alleyway apparatus. The numbers 1–7 indicate locations consisting of exchangeable acrylic plates.

2. Methods

2.1. Animals

Seventy-one, 3–4 month-old, male Wistar rats were used. The animals were raised in litters reduced to six pups, and weaned after 25 days. Groups of 2–3 rats were then randomly housed in wire cages measuring 30 cm × 16 cm × 18 cm, with a 1-cm grid spacing. Light was provided on a 12-h light:12-h dark cycle (lights on at 7:00 a.m.) and room temperature was kept at 23 °C ± 2. These housing conditions were maintained until the experiments ended.

2.2. Apparatus

The alleyway apparatus was constructed of acrylic, and consisted of two, 30 cm × 30 cm × 30 cm boxes connected by 8 cm × 8 cm guillotine doors to the opposite ends of a straight alley 100 cm long, 14 cm wide and 30 cm high (Fig. 1). Each of the boxes contained a 3-cm-diameter by 8-mm-deep hole in the floor, 4.5 cm distant from the wall opposite the door, in which three sunflower seeds were offered as a reward. The guillotine doors could be operated from a distance by the experimenter, using nylon thread. The walls and the floor were white. The floor consisted of seven, 13-cm long acrylic plates intercalated with eight, 1.5-cm-wide rails into which the plates were inserted like drawers. According to its position in the alleyway, each plate defined a location (Fig. 1). One or several of these plates could be replaced by other equal-sized black or black and white checkered plates (which provided visual stimuli since they differed from the rest of the floor). Photocells were placed on the walls aligned with the rails and were connected to a microcomputer, permitting measurement of the time the rats spent at each location in the alleyway.

2.3. Food deprivation

The animals were kept on a food deprivation schedule with 60-min daily access to food; special care was taken to ensure that the animals maintained at least 85% of their initial body weight. This schedule lasted until the experiments ended.

2.4. Statistical analysis

A non-parametric analysis of variance for repeated measures (Friedman's Test) complemented by a Multiple Comparisons Test based on rank sums [8] was used to compare the time spent in different trials at the various locations of the alleyway. A non-parametric analysis of variance (Kruskal Wallis' Test) was used to compare groups; a single analysis was performed for each comparison. Since the time spent at the different locations in the alleyway did not follow a normal distribution, limiting the use of parametric descriptors, the median time was chosen to express the data. Only values of $p \leq 0.05$ were considered significant.

3. Behavioral procedures

3.1. Handling

For 4 days prior to pre-exposure to the apparatus, the animals were handled for 30 s per day and received sunflower seeds to become accustomed to this food source used later as reward.

3.2. Pre-exposure to the apparatus and training

Each animal was pre-exposed for 10 min to the apparatus. Both guillotine doors were opened, and the rats were allowed to explore the entire maze.

During the training period which started on the following day, each animal was submitted to one training session per day, with eight trials per session. For the first trial of each session, the rat was placed in the box near Place #1 (see Fig. 1). After 10 s, the guillotine doors were opened, allowing the animal to move to the other box which was baited with three sunflower seeds. After the animal had entered this box, the door was closed. When the animal had consumed the seeds, the doors were re-opened, allowing the rat to return to the original box, now also baited with three sunflower seeds (second trial). Thus, the trials within each session consisted of four trials in one direction and four in the opposite direction. The time the animals spent at each location in the alleyway was measured by the computer. When an animal failed to reach the goal box within 180 s, it was replaced in its cage, finishing the training session for that day. During the 10-day training period, all seven floor plates were white. On day 11, drug administration was begun in association with the testing schedule (see below).

3.3. Testing phases

For Test Phase 1, a black plate was introduced at Place #2 in Trials 4, 6 and 8; for the remaining trials (1–3, 5 and 7), a white plate was inserted at this location. Note that the black plate stimulus was introduced intermittently, at the same location, and always when the animal was moving in a specific direction. The data for trials using stimulus presentation (4, 6 and 8) were compared to those for Trial 2 (in which the direction of movement was the same as that in the trials with stimulus presentation, although without a stimulus present). This within-subjects comparison, using Trial 2 as a reference for the animals' activity level during the same session in which testing to stimulus change was performed, represents the best baseline possible. Since each animal is tested within the same behavioral, contextual and drug condition as those present in the testing trials (4, 6 and 8), it constitutes the best control of its own performance (see Xavier et al. [29]). Further, Trial 2 data (the baseline prior to stimulus change) for the different groups were also compared. Thus, this between-subjects comparison furnishes information on the impact of the different treatments on the baselines of the different groups. This procedure was maintained for two further sessions as required until habituation of the response to stimulus presentation. In addition, training sessions similar to those described above in which no stimulus was presented to the rats were interspersed among these testing and habituation sessions in which the black plate stimulus was presented. These training sessions interspersed with the testing sessions controlled for the development of tolerance to the drug associated with performance of the task. That is, this schedule allowed exposure of the rats to the drug treatment associated with moving through the alleyway, but not to stimulus presentation.

During Test Phase 2, the same black plate was now introduced to Place #6 in Trials 4, 6 and 8, for 2 days, and during the corresponding interspersed training sessions no stimulus was presented (see below). Thus, the first day of Test Phase 2 corresponded to a change in the location of stimulus presentation.

When the animals no longer reacted to the intermittent presentation of the black plate at Place #6 (habituation had occurred), their ability to react to a change in the stimulus itself (the black plate stimulus was substituted by a black and white checkered plate stimulus, presented in Trials 4, 6 and 8) was evaluated in the subsequent session.

3.4. Drug treatments

From Test Phase 1 on, each rat received an intraperitoneal (i.p.) injection of either 24 mg/kg atropine sulfate or 0.9% saline, 20 min before the beginning of each session.

The schedule of drug administration followed a 2 × 2, state-dependent, learning experimental design, including the pairings N-N, N-D, D-N and D-D, both when the animal was exposed to a change in the location of stimulus presentation (*i.e.*, from Test Phase 1 to Test Phase 2; see below) and when it was exposed to a change in the visual pattern of the stimulus (*i.e.*, from Test Phase 2 to Test Phase 3; see below). The detection of these changes respectively required comparison between a previous location of stimulus presentation with the current location, and comparison between a previously presented visual stimulus and the current stimulus. Thus, it was possible to compare state dependency for the detection of spatial change, and for the detection of stimulus change.

The possible occurrence of behavioral and/or pharmacological tolerance was controlled; the behavioral and drug administration schedules were such that the number of atropine injections was the same for all critical groups before each testing phase. Thus, testing and habituation sessions with stimulus presentation were interspersed with training sessions without stimulus presentation. While groups that received atropine during the testing sessions (with stimulus presentation) received saline during the interspersed training sessions (without stimulus presentation), other groups received atropine during the interspersed training sessions and saline during the testing sessions. This schedule equalized the number of atropine injections in all groups before each testing phase. Thus, in Test Phase 1, independent groups of animals received (1) saline before the testing and habituation sessions in which they were exposed to stimulus presentation, and atropine during the interspersed training sessions in which they were not exposed to stimulus presentation, but were exposed to the eight trials in the alleyway task under the drug effect; thus, they could also develop behavioral and/or pharmacological tolerance without being exposed to the critical stimulus ($N = 18$). Performance of this group was later (during Test Phase 2) compared to that of (2) another group that received atropine during the testing and habituation sessions with stimulus presentation, and saline during the interspersed training sessions without stimulus presentation ($N = 24$). When these groups were later submitted to administration of either saline or atropine during Test Phase 2, taking into account the state-dependent learning schedule for drug administration, their previous experiences with atropine were equivalent, even though for the first group exposure to the critical novel stimulus took place under a saline effect, while for the second group, exposure to the stimulus occurred under an atropine effect. A third group (3) received saline during both the testing and habituation sessions, and during the interspersed training sessions ($N = 29$), thus corresponding to a control group for the potential development of tolerance by the other groups.

A similar control schedule was used for the transition between Test Phases 2 and 3.

4. Test Phase 1. Effects of atropine on the reaction to the presentation of a novel stimulus in the alleyway task

By the 10th training session the animals had reached an asymptotic level of running performance. They were then divided into two matched-for-acquisition-curve groups, one to be injected with atropine (47 rats) and the other with saline (24 rats) before each of the testing and habituation sessions which began on day 11.

Each rat received either atropine (A) or saline (S) 20 min before beginning the test session; in these sessions, a black plate stimulus was introduced at Place #2, in Trials 4, 6 and 8; in the remaining trials (1–3, 5 and 7) the usual white plate used during training was presented at Place #2. Thus, the stimulus was introduced intermittently, and always when the animal was moving in a specific direction, and always at the same location. This procedure was maintained for two additional habituation sessions as required until complete habituation of the response to stimulus presentation. In addition, training sessions in which no stimulus was presented to the rats were included to control for pharmacological and behavioral tolerance (see above).

4.1. Results and discussion

Fig. 2 shows the response to the location at which the stimulus was presented in Trials 4, 6, and 8 for Test Phase 1, and for Trial 2, without stimulus presentation used here as a refer-

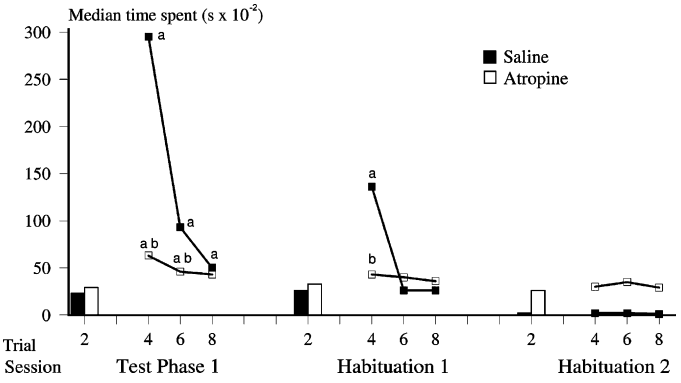

Fig. 2. Response to stimulus presentation expressed as the median time spent at Place #2 in Trials 2 (no stimulus presentation), 4, 6 and 8 (stimulus presentation) during Test Phase 1 sessions for the groups injected with saline and atropine. (a) $P < 0.05$ compared to Trial 2 (Friedman's Test and Multiple Comparisons Test); (b) $P < 0.05$ compared to the saline-injected group (Kruskal Wallis Test).

ence for the groups that received either atropine or saline during testing with stimulus presentation. The introduction of a black plate at a specific location in the alleyway in Trials 4, 6 and 8 produced a significant increase in the time animals spent exploring the novelty at that location compared to the time spent at the same location in the absence of stimulus presentation (Trial 2) (Friedman's Test, group injected with saline, $X^2 = 160.54$, $P < 0.01$, group injected with atropine, $X^2 = 28.01$, $P < 0.05$). A *post hoc* Multiple Comparisons Test revealed that for saline-injected animals, the amount of time spent at Place #2 in trials with stimulus presentation was greater than that in Trial 2 without stimulus presentation (see relevant statistical comparisons in Fig. 2), indicating, as expected, that saline-injected rats did detect and react to the novelty. Atropine-injected rats also reacted to the novelty as shown by the increase in time spent at Place #2 in Trials 4 and 6, compared to the time they spent at Place #2 in Trial 2 (see relevant statistical comparisons in Fig. 2). However, this response was reduced compared to that seen for saline-injected rats (Fig. 2); the Kruskal Wallis test showed that the time spent at Place #2 for saline- and atropine-injected rats differed significantly between Trials 4 and 6 in Test Phase 1 ($H(1) = 6.45$, $P < 0.05$), and in Trial 4 of the first Habituation session ($H(1) = 4.02$, $P < 0.05$) (see Fig. 2). Together, these results suggest that although atropine did not completely disrupt the reaction to novelty it did interfere with this response, possibly by affecting exploratory activity towards novelty. Importantly, the Kruskal Wallis Test did not reveal statistical differences between the saline- and atropine-injected group scores in Trial 2 (without stimulus presentation) ($H(1) = 1.34$, $P > 0.05$), indicating that atropine does not interfere with the locomotor activity required for performance of this task, in the dose used.

The intermittent presentation of this stimulus at the same location led to a decrease in this response both in the saline- and atropine-injected rats (Fig. 2, Habituation sessions 1 and 2).

Lipp and Schwegler [15] have reviewed the extensive literature showing that the size of the infra-intra-pyramidal mossy fibers (IIP-MF) in the rodent hippocampal formation may vary considerably as a function of genetic factors and experimental manipulation. Most interestingly, the size of this projection and the performance of rodents in a diversity of behavioral tasks are correlated. For example, the size of the projection is positively correlated with performance in the Morris' water maze and in the 8-arm radial maze; in contrast, there is a negative correlation between the size of the projection and performance in the two-way active avoidance task. According to Lipp and Schwegler [15], the greater the size of the IIP-MF, the greater the regularity of the animal's behavior (termed "behavioral predictability" by these authors) since the balance between the influence of the supra-pyramidal mossy fibers projection (SP-MF) and the IIP-MF on the CA3 pyramidal cells becomes altered. Blockade of cholinergic transmission within the hippocampus may alter this balance leading to greater behavioral predictibility and, therefore, diminished distractibility, manifested as the probability of the individual to interrupt ongoing behavior and re-direct attention towards a novel stimulus. Alternatively, the animals may re-direct attention towards the source of novelty but explore it less extensively than do control rats injected with saline. Test Phase 2 investigated these hypotheses by testing the animals' reaction to a change in the location of stimulus presentation following atropine or saline injection.

5. Test Phase 2. Change in the location of stimulus presentation in association with a change in drug state

When the animals no longer reacted to the intermittent presentation of a black plate at Place #2 (habituation had occurred), their ability to react to a change in the location of stimulus presentation (the same stimulus now presented at Place #6 in Trials 4, 6 and 8) was evaluated in association with a change in drug state, following the state-dependent learning schedule of drug administration. Thus, animals first subjected to Test Phase 1 under the drug (D) and no-drug (N) effects were now subjected to Test Phase 2 to evaluate their reaction to spatial change under the effect of either the same drug (and, therefore, in the same "drug state") or in a different state, according to the state-dependent learning schedule N-N, N-D, D-N and D-D. Three additional groups were submitted to the conditions N-N, N-D and D-N in association with presentation of the same stimulus at the same location as in Test Phase 1; *i.e.*, no spatial change was introduced for these three groups so as to control for possible state-dependent effects related to habituation, and reaction to the stimulus itself.

Table 1 provides details of the resulting groups and corresponding drug and behavioral manipulations.

As seen in Table 1, the animals in group S2-S6(A) were injected with saline (S) during Test Phase 1 (stimulus at Place #2), with atropine given during the interspersed training sessions without stimulus presentation, and with saline during Test Phase 2 (stimulus at place #6). Thus, these animals were exposed to a change in location of stimulus presentation together with an N-N condition, but were given atropine during the interspersed training sessions to provide the opportunity for the development of pharmacological and behavioral tolerance to the drug effect. For group S2-A6(A), injected with saline during Test Phase 1 when the stimulus was presented at Place #2, and with atropine during Test Phase 2 when the stimulus was presented

Table 1
Groups and corresponding drug and behavioral manipulations performed in Test Phase 1 (introduction of a novel stimulus) and Test Phase 2 (spatial change for stimulus presentation and control condition without a spatial change)

Group	*N*	Test Phase 1	Test Phase 2	State dependent condition (testing)
S2-S6 (A)	18	Saline	Saline	N-N (Spatial change)
S2-A6 (A)	10	Saline	Atropine	N-D (Spatial change)
A2-S6 (S)	8	Atropine	Saline	D-N (Spatial change)
A2-A6 (S)	8	Atropine	Atropine	D-D (Spatial change)
S2-S2 (S)	9	Saline	Saline	N-N (No spatial change)
S2-A2 (S)	10	Saline	Atropine	N-D (No spatial change)
A2-S2 (S)	8	Atropine	Saline	D-N (No spatial change)

The numbers included in the group names indicate the place location for stimulus presentation in Test Phase 1 and Test Phase 2, respectively. Letters (A, atropine and S, saline) represent the substance injected during the Test Phases and the interspersed training sessions (between parentheses in the respective identifications).

at Place #6, spatial change was to be detected together with the injection of atropine (condition N-D, and therefore, under a drug state change), in animals which had already experienced atropine during the interspersed training sessions without stimulus presentation. An additional control group, S2-A2(S), was given saline during Test Phase 1 (stimulus at Place #2), also given saline during the interspersed training sessions without stimulus presentation, and received atropine during Test Phase 2 (in this group, the stimulus was presented at Place #2). Thus, this group was not exposed to a spatial change; since atropine was injected before Test Phase 2, but the stimulus was presented at the same location as before, the experiment evaluated whether atropine promotes dis-habituation to stimulus presentation. Another control group, S2-S2(S), involving saline injection during both Test Phases and no spatial change, evaluated the effect of an additional habituation session to stimulus presentation at the same location in the absence of a drug effect. In contrast, animals in group A2-S6(S) were given atropine during Test Phase 1 (stimulus at Place #2), saline during the interspersed training sessions, and saline during Test Phase 2 (stimulus at Place #6); these animals were exposed to a change in the location of stimulus presentation together with a D-N condition. Thus, they became habituated to stimulus presentation at Place #2 under the atropine effect but were also tested to examine the spatial change without the atropine effect. Since the group A2-A6(S) received atropine before both Test Phase 1 and Test Phase 2, this experiment allowed evaluation of the rats' ability to detect the spatial change under the same drug state as that in which habituation occurred. To evaluate a possible dis-habituation effect associated with the drug state change (D-N), a third group, A2-S2(S), received atropine during Test Phase 1 and saline during Test Phase 2, without change in the location of stimulus presentation.

In short, (1) group S2-S6 was expected to show the usual reaction to spatial change compared to the lack of spatial change in group S2-S2, (2) group S2-A6 allowed evaluation of the effect of atropine on the detection of spatial change, having group S2-A2 as a control for a possible atropine dis-habituation effect by the stimulus, which itself requires group S2-S2 as a control, and (3) group A2-S6 allowed evaluation of the ability to detect a spatial change together with a drug state change, having group A2-A6 as a control for the drug state change and group A2-S2 as a control for the change in stimulus presentation in association with the drug state change.

5.1. Results and discussion

Fig. 3 shows the time spent at the location where the stimulus was presented in Trials 4, 6 and 8, and in Trial 2, without stimulus presentation (used as a reference control) in Test Phase 2.

It should be noted, firstly, that the magnitude of the animals' response to spatial change (Fig. 3) was reduced compared to that following the introduction of a novel stimulus in the alley-way (Fig. 2, above, and Fig. 4, below), and thus, different scales have been used to present the data. These results reproduce previous observations from our laboratory (Xavier et al. [29,30])

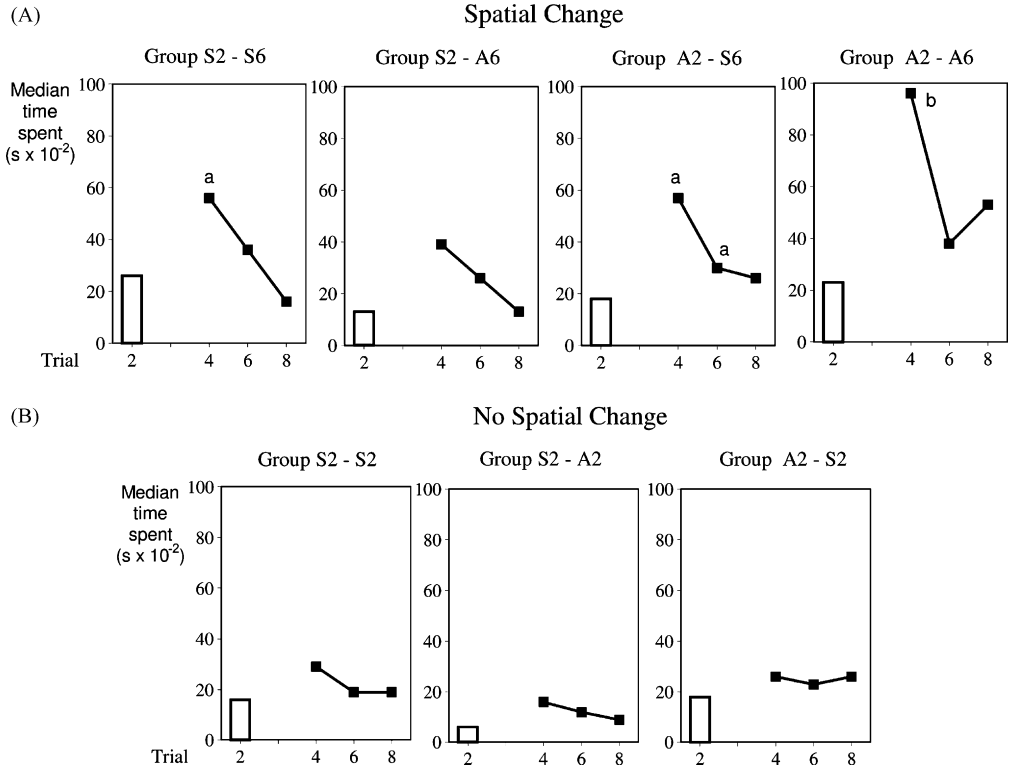

Fig. 3. Response to stimulus presentation expressed as the median time spent at Place #6. (3A: groups exposed to a change in the location of stimulus presentation) and at Place #2 (3B: groups not exposed to a change in the location of stimulus presentation) in Trials 2 (no stimulus presentation), 4, 6 and 8 (with stimulus presentation), during the first session of Test Phase 2. A, atropine and S, saline. (a) $P < 0.01$ relative to respective Trial 2 (Friedman's Test and Multiple Comparisons Test). (b) $P < 0.05$ relative to respective Trial 2 (Friedman's Test and Multiple Comparisons Test).

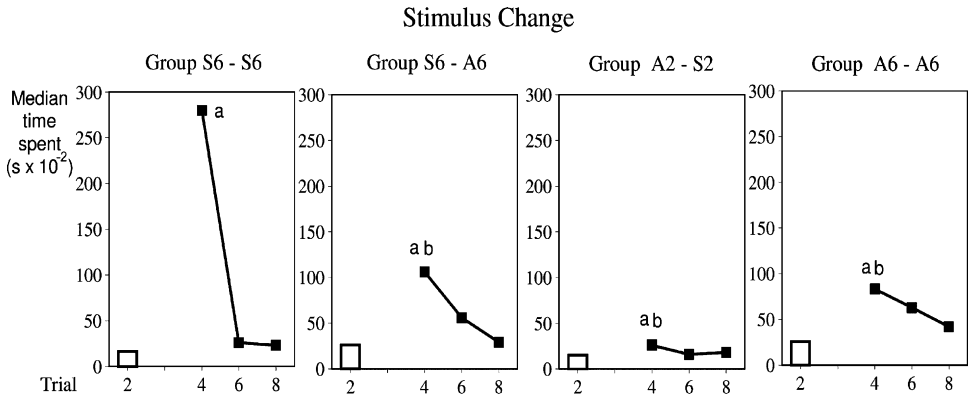

Fig. 4. Response to novel stimulus presentation expressed as the median time spent at Place #6 (groups S-S, S-A and A-A) and Place #2 (group A-S) in Trials 4, 6 and 8 (stimulus presentation), and in Trial 2 (no stimulus presentation), during the first session of Test Phase 3. A, atropine and S, saline., (a) $P < 0.05$ relative to respective Trial 2 (Friedman's Test and Multiple Comparisons Test), (b) $P < 0.05$ relative to group S6-S6 (Kruskal Wallis' Test and Multiple Comparisons Test).

showing that reaction to the presentation of a novel stimulus in the alleyway is greater compared to that following a change in the location of an already familiar stimulus. Confronting similar findings, Xavier et al. [29] noted "*. . .that the magnitude of the response* (to the spatial change of a familiar stimulus) *was lower than that resulting from the introduction of a new stimulus. . ., suggesting the validity of the distinction between the response to the qualitative properties of the stimulus and the context of its presentation (O'Keefe and Nadel, 1978). In other words, the novelty here is related to the change in the configuration of the familiar stimuli and not to the presentation of a stimulus with novel properties"* (p. 166).

Despite this generally diminished reaction to spatial change, Friedman's Test revealed that groups S2-S6 ($X^2 = 37.56$, $P < 0.01$), A2-S6 ($X^2 = 28.67$, $P < 0.01$) and A2-A6 ($X^2 = 16.95$, $P < 0.05$), which were exposed to a change in the location of stimulus presentation, significantly increased the amount of time spent at the new location of stimulus presentation. Differently, groups S2-S2 ($X^2 = 5.111$, $P > 0.05$), S2-A2 ($X^2 = 6.328$, $P > 0.05$) and A2-S2 ($X^2 = 7.366$, $P > 0.05$), which were not exposed to a change in the location of stimulus presentation, showed no statistical difference between the time spent at the location of stimulus presentation in Trial 2 (without stimulus presentation), and in Trials 4, 6 and 8 (stimulus presentation) (see Fig. 3 for relevant statistical comparisons). Thus, groups S2-S6, A2-S6 and A2-A6 detected the spatial change; the *post hoc* Multiple Comparisons Test showed that the scores for Trials 2 and 4 differed significantly for all these groups (see relevant statistical comparisons in Fig. 3). Thus, animals subjected to the N-N (S2-S6), D-D (A2-A6) and D-N (A2-S6) schedules of drug administration, as well as to the spatial change, reacted to this latter manipulation, suggesting that they compared the information previously acquired concerning the location at which the stimulus had been presented during Test Phase 1 with the information on the new location of stimulus presentation (Test Phase 2). Note that groups A2-A6 and A2-S6 acquired this spatial information under an atropine effect; even so, they were able to compare this information with the new information, both under the atropine effect (Group A2-A6) and without the atropine effect (Group A2-S6), enabling response to the change in location of stimulus presentation. Differently, Friedman's Test revealed no statistical difference for group S2-A6 ($X^2 = 7.302$, $P > 0.05$), indicating that these rats have difficulty in comparing spatial information acquired in the absence of the atropine effect with novel (and different) spatial information acquired under the atropine effect. We emphasize that the reaction of group A2-A6 to the spatial change did not differ from that shown by groups S2-S6 and A2-S6 ($H(3) = 7.374$, $P > 0.05$), which received saline before testing for the reaction to spatial change; apparently, atropine does not disrupt the ability to re-direct attention towards a source of novelty and, in addition, does not interfere with the ability to interrupt the ongoing running response for food and to explore the source of novelty (in this case the spatial change), when the previous spatial information was acquired under the same drug effect. Further, the lack of reaction by group S2-A2 in Test Phase 2 suggests that atropine does not disrupt the animals' ability to identify the stimulus presented, since otherwise, these rats would have reacted to the change. Together, these findings suggest that atropine disrupts the retrieval of spatial information acquired during normal hippocampal function.

The state-dependent effect seen for animals exposed to the N-D condition seems to be specifically related to the spatial change; the animals in group S2-A2, which were also subjected to the N-D schedule but not to the spatial change, exhibited the same pattern of results, *i.e.*, showed no increase in the time spent at the location of stimulus presentation. Thus, there seems to be no state-dependent learning effect regarding the identification of a previously presented stimulus. Test Phase 3 addressed this question explicitly.

6. Test Phase 3. Change of visual stimulus in association with a change in drug state

Xavier et al. [29] showed that the presentation of a novel visual stimulus in the alleyway test, after habituation of rats to the intermittent presentation of a different visual stimulus presented previously, leads to exploratory activity directed towards the source of novelty. Xavier et al. [30] showed that dorsal hippocampal damage does not interfere with this reaction, suggesting that these rats are able to compare the representation of

Table 2
Groups and corresponding drug and behavioral manipulations performed in Test Phase 2 (spatial change) and Test Phase 3 (visual stimulus change)

Group	N	Test Phase 2	Test Phase 3	State dependent condition (testing)
S6-S6 (A)	9	Saline	Saline	N-N (Stimulus change)
S6-A6 (A)	9	Saline	Atropine	N-D (Stimulus change)
A2-S2 (S)	10	Atropine	Saline	D-N (Stimulus change)
A6-A6 (S)	10	Atropine	Atropine	D-D (Stimulus change)

The numbers included in the group names indicate the location of stimulus presentation in Test Phase 2 and Test Phase 3, respectively. A, atropine and S, saline, correspond respectively to the substance injected before each Test Phase.

the previous stimulus stored in memory with that of a current stimulus, and to detect and react to the difference.

Test Phase 2 showed that the rats' reaction to spatial change is disrupted when the animals are concurrently exposed to a drug state change according to the N-D condition, suggesting that atropine interferes with the retrieval of spatial information acquired in the absence of the drug effect. Test Phase 3 evaluated whether the same effect occurs in relation to a change in visual stimulus. The animals in groups S2-S2, A2-S2, A2-S6 and A2-A6 were not included in this Test Phase.

When the animals in the remaining groups no longer reacted to the intermittent presentation of the black plate at Place #6 in Test Phase 2, *i.e.*, habituation had occurred, their ability to react to a change in the stimulus itself (the black plate was substituted by a black and white checkered plate, presented in Trials 4, 6 and 8), was evaluated in association with the state-dependent learning schedule of drug administration. That is, animals first subjected to Test Phase 2 were now subjected to Test Phase 3 to evaluate their reaction to the visual stimulus change under the state-dependent learning conditions N-N, N-D, D-N and D-D.

Table 2 provides the resulting groups and corresponding drug and behavioral manipulations.

6.1. Results and discussion

Fig. 4 shows the median time spent at the location at which the novel stimulus was presented in Trials 4, 6 and 8, and in Trial 2 (without stimulus presentation, used as reference control), during Test Phase 3.

Friedman's Test showed that all groups reacted to stimulus change [S6-S6 ($X^2 = 33.08$, $P < 0.01$), S6-A6 ($X^2 = 16.29$, $P < 0.05$), A2-S2 ($X^2 = 19.35$, $P < 0.05$) and A6-A6 ($X^2 = 22.24$, $P < 0.05$)]. A Multiple Comparisons Test revealed that these differences involved the time spent at the location of stimulus presentation in Trial 2 (without stimulus presentation) compared to Trial 4 (with stimulus presentation) (see relevant statistical differences in Fig. 4). Thus, all groups were able to detect and react to the novel visual stimulus, directing exploratory activity towards the location at which it was presented; however, the extent of this reaction varied considerably among the groups, as shown in Fig. 4. In fact, the Kruskal Wallis non-parametric analysis of variance showed significant differences in group reactions to the novelty in Trial 4 ($H(3) = 15.326$, $P < 0.05$); the *post hoc* Multiple Comparisons Test revealed that the time spent at the location of stimulus presentation by group S6-S6 (N-N) was significantly greater compared to the corresponding scores of groups S6-A6 (N-D), A2-S2 (D-N) and A6-A6 (D-D) which, in turn, were not significantly different among each other (see Fig. 4 for relevant statistical comparisons). Differently, the Kruskal Wallis Test revealed a lack of significant group differences in Trial 2 (when no stimulus was presented) ($H(3) = 6.925$, $P > 0.05$), indicating that the atropine injection did not disturb the rats' running response for food when no stimulus was presented in the alleyway test.

Thus, the administration of atropine before the introduction of a novel visual stimulus (groups S6-A6 and A6-A6), decreased, but did not preclude, the reaction to this novelty. Together with data from Test Phase 1, these results show that rats under an atropine effect are able to re-direct their attention towards the source of a non-spatial novelty but do not explore it as extensively as do saline-injected rats. In contrast, the results of Test Phase 2 show that when the novelty involves a spatial change, the rats under an atropine effect are unable to detect and react to it, unless they have acquired a prior spatial configuration to compare with the current one under the atropine effect (Test Phase 2); this result corroborates the view that atropine changes the way information is stored, rendering its availability greater when a similar drug state is installed. The decreased reaction to stimulus change seen in the present Test Phase by groups A2-S2 and A6-A6 provides additional support for this interpretation.

7. General discussion

The probability of retrieving previously acquired information seems to increase when the brain's physiological state and the sensory context at the time of original acquisition are at least partially reinstated at the time of testing. Thus, if information is acquired under the effect of a specific drug that alters brain functioning, it is probable that its retrieval is increased by administration of the same drug previously to the retrieval session. This phenomenon termed "state-dependent learning", is robust and has been described for a diversity of behavioral tasks and drugs ([9,12,20]).

It is well known that atropine-like drugs tend to produce state-dependent learning [21]. Thus, in the present study, atropine was used to investigate whether state-dependent learning is related to the nature of the information being processed. Our experimental design associated the state-dependent learning paradigm for drug administration [21] and behavioral testing in the straight alleyway test [24,25]; both spatial, and visual, non-spatial information were tested. One of the advantages of using this behavioral task is the detection of both spatial and non-spatial changes results in similar exploratory responses; thus, as long as the animals react to either the spatial or the stimulus change, possible sensory and motor effects of the drug can be discarded.

During Test Phase 1, when a new stimulus (black plate) was introduced into the alleyway, both saline- and atropine-injected animals reacted to the novelty, directing their exploratory activity towards the location where the stimulus had been placed. However, the time spent exploring the novelty was less for the

atropine-injected rats (Fig. 2). During Test Phase 2, when a spatial change was introduced into the alleyway together with the N-N, D-D and D-N schedules of drug administration, the animals significantly increased the time spent at the novel location (Fig. 3), indicating that they reacted to the spatial change. Differently, however, animals subjected to the N-D schedule did not significantly increase the time spent at the novel location (Fig. 3), suggesting that they may be unable to compare spatial information acquired in the absence of an atropine effect with current (and different) spatial information presented under an atropine effect. Interestingly, the animals exposed to the spatial change under atropine, after habituation to the stimulus under the same drug state (Group D-D), did increase the time spent at the novel location (Fig. 3), indicating that these animals could compare and react to spatial change. Together, these findings show that atropine disrupts the retrieval of spatial information acquired in the absence of the drug, *i.e.*, during normal functioning, and also that atropine alters the nature of the information stored, rendering retrieval more likely when the animal is under the same drug state (condition D-D). Further, the experimental design used in this study shows that these findings are unrelated to either the pharmacological or behavioral development of tolerance, since the animals in all groups were subjected to testing after having received the same number of drug injections associated with behavioral training in the alleyway.

Sala et al. [23] trained rats in the 8-arm radial maze, a task usually considered to require spatial orientation, without a drug effect, and then investigated the effect of intra-cerebroventricular injections of the anticholinergic drugs, atropine and pirenzepine, on task performance. Their results show that increasing doses of atropine and pirenzepine significantly impaired task performance. Similarly, Fraser et al. [5] found that the performance of rats previously trained in a spatial matching to sample (MTS) task is disrupted when they are tested under an atropine effect. Congruently, Schulze et al. [24] trained monkeys in an operant test battery involving spatial, temporal and/or complex association, and then tested the animals under an atropine effect. Their data show a dose-dependent disruption of performance in tests involving all kinds of information. Thus, the common finding emerging from these studies concerns the interference of anti-cholinergic drugs with the performance of spatial and/or complex tasks previously acquired without the drug effect. Note, however, that these data do not allow exclusion of hypotheses regarding the presence of a state-dependent learning effect or a drug-induced sensory and/or motor change that may have interfered with performance.

The present experiments were designed to allow evaluation of these hypotheses. In Test Phase 2, atropine promoted state-dependent learning related to the access to spatial information in the straight alleyway test; that is, atropine disrupted the reaction to a spatial change when administered previously to the introduction of the novelty (schedule N-D), but not when administered in the D-D schedule. Together, these results indicate that the exposure to a stimulus at a specific location without an atropine effect renders this information difficult to retrieve when under an atropine effect. However, since the animals in group D-D, like the controls, reacted to the spatial change under the atropine effect, (1) atropine does not interfere with the sensory and motor functions required for detection and reaction to novelty in this task, and (2) spatial information acquired under the atropine effect is properly retrieved following injection of the same drug.

Favoring this interpretation, Richter-Levin and Segal [22] showed that performance in a reference memory version of the water maze task, which requires spatial memory, is not altered when atropine is injected during task acquisition. Note that the daily administration of atropine, as in Richter-Levin and Segal's [22] study, is comparable to the D-D schedule used in the present study, and that in both studies the animals were capable of making use of the spatial information despite being under an atropine effect.

During Test Phase 3, the animals reacted to the visual stimulus change, irrespective of changes in drug state, indicating that atropine does not promote state-dependent learning when the nature of the current information to be compared to that stored in memory is not spatial.

Similarly, Xavier et al. [30] showed that dorsal hippocampectomy completely disrupts the reaction to a change in the location of stimulus presentation but does not interfere with the rats' ability to react to a visual stimulus change. In the present experiments, atropine injection reduced, but did not preclude, the animals' reaction to stimulus change. This discrepancy may be ascribed to a greater dysfunction induced by systemic injections of atropine compared to minute dorsal hippocampectomy. Alternatively, atropine-injected rats may detect the change but are unable to interrupt their ongoing response so as to explore this novelty. In favor of this interpretation, Flicker and Geyer [4] showed that intra-hippocampal infusions of atropine induce a persistent pattern of locomotor activity, while Monmaur, Sharif and M'Harzi [17] showed that the septo-hippocampal system plays a critical role in the release of exploratory behavior.

Together, the present findings demonstrate, apparently for the first time, that atropine disrupts the retrieval of spatial information, but not of non-spatial, visual stimulus information, indicating that state-dependence may depend upon the nature of the information being retrieved, and thus on the underlying brain system.

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